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Small Molecule Inhibitors of EGFR Ectodomain for Breast Cancer Therapy

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of this proposal was to develop low molecular weight inhibitors of EGFR that disable receptor functioning by preventing critical activating transitions of the extracellular domain. A compound, EL1-FD1, has been created and found able to reverse the malignant properties of EGFR transformed cells *in vitro* and *in vivo*. EL1-FD1 was designed to limit the mobility of the EGFR ectodomain, in particular subdomains I and II. For Task 3a, we have optimized therapeutic administration of EL1-FD1 in animal tumor bearing models. For Task 3b, we have analyzed the effects of the compound *in vitro* in anchorage dependent and independent assays. None of the analogs we identified in Task 4 possessed greater activity than EL1-FD1. For Task 5 we have studied features of how the constrained EGFR binds ligand. The ability of the compound that constrains EGFR mobility to bind EGF was found to be dramatically reduced. We have not yet succeeded in co-crystallization as described in Task 6 and still are pursuing different conditions for formation of crystal structures of the complex.

15. SUBJECT TERMS
EGFR inhibitor, antitumor activity

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INTRODUCTION.

Since EGFR is overexpressed in many breast cancers, inhibition of this receptor is a promising approach for the development of targeted breast cancer therapeutics. The kinase-targeting low molecular weight EGFR inhibitors used in clinics have been shown to have some activity in patients with mutant EGFR kinase, but have essentially no activity in patients with wild type EGFR. We used structure based approaches to design low molecular weight compounds targeted to the ectodomain of EGFR that act by inhibiting conformational rearrangements required for receptor activation. During the reported period, the lead compound EL1-FD1 and its derivatives have been tested for inhibition of breast cancer cells *in vitro* and *in vivo*. We also continued the lead optimization and crystallography studies.

BODY.

In vivo studies Task 3 – Use of EL1-FD1 *in vivo*. In the past year we have identifed ways to improve the solubility of ELI-FD1 for *in vivo* application. We examined the activity of EL1-FD1 in an aggressive human tumor driven solely by EGFR over-expression. This small molecule has extraordinary activity *in vivo*. The EL1-FD1 small molecule has a Mr of ~350 and completely arrests growth of A431 tumor cells *in vivo* but has no activity against tumor cells which are not transformed by EGFR.

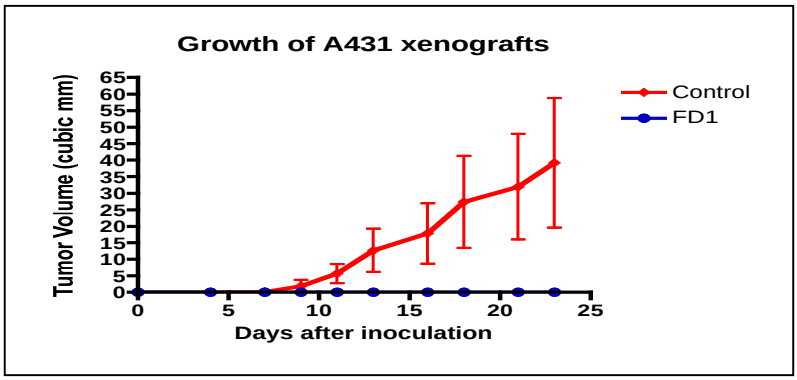

Figure 1. Growth of A431 tumor cells is dramatically inhibited by EL1-FD1. Athymic nude mice were injected s.c. with $1x10^6$ A431 cells on the flank. EGFR inhibitor EL1-FD1 was administered by i.p. injection at a dose of 15 mg/kg, three times per week beginning on day 4. The data are presented as mean tumor volume (mm3) ±SEM.

Task 4. We obtained several analogs of the EL1-FD1, but none to date have improved biological activities. New analogs have been designed and will be tested *in vitro* and *in vivo*.

Task 6. We have tried to soak the EGFR ectodomain crystals with EL1-FD1 but have not been able to develop a complex crystal. This failure may relate to the conditions of the soaking and the solubility features of the EL1-FD1 molecule, and we are now working towards co-crystallization of EL1-FD1 and EGFR. To facilitate crystallization, more water-soluble analogs of EL1-FD1 with similar biological activities to the parent compound have been designed and will also be used in crystallography studies. These studies are ongoing.

KEY RESEARCH ACCOMPLISHMENTS.

We have extended the biological studies on EL1-FD1 to A431 tumors driven solely by EGFR and have observed dramatic and complete inhibition of tumor growth *in vivo*.

We have not succeeded in developing co-crystals using soaking of the somewhat insoluble EL1-FD1 and we are now working on co-crystallization studies of the protein and the small molecule together and on the improvement of the compound solubility to facilitate crystallization of the protein-compound complex.

REPORTABLE OUTCOMES.

A manuscript describing the obtained results is in preparation.

CONCLUSION.

The overall goal of this proposal was to develop low molecular weight inhibitors of EGFR that disable receptor functioning by preventing critical activating transitions of the extracellular domain. The compound we term EL1-FD1 has been created and found able to reverse the malignant properties of EGFR transformed cells *in vitro* and *in vivo*. We have extended the studies to other tumors to verify their activity and surprisingly have found even more complete tumor eradication of EGFR driven A431 tumor lines. The compound was designed to limit the mobility of the EGFR ectodomain, in particular subdomains I and II. Since the last report we have focused on Task 3a, and we have now optimized therapeutic administration of EL1-FD1 in animal tumor bearing models. Moreover, as defined in Task 3b, we have analyzed the effects of the compound *in vitro* in anchorage dependent and independent assays. We have already shown in Task 3c that EL1-FD1 affects specific tyrosine kinase activation events. Task 4 was to improve on the chemical features of EL1-FD1 by selection of new analogs. This task has not progressed in this time frame, as none of the analogs we identified possessed greater activity than EL1-FD1. As described in Task 5 we have studied certain features of how the constrained EGFR binds ligand. We tested the ability of the compound that constrains EGFR mobility to bind EGF and found it to be dramatically reduced. We have not yet succeeded in co-crystallization as described in Task 6 and still are pursuing different conditions for formation of crystal structures of the complex.